TRANSLATION

I, Yuko Mitsui, residing at 4-6-10, Higashikoigakubo, Kokubunji-shi, Tokyo, Japan, state:

that I know well both the Japanese and English languages,

that I translated, from Japanese into English, Japanese Patent Application No. 2000-087501, filed on March 27, 2000, and

that the attached English translation is a true and accurate translation to the best of my knowledge and belief.

Dated: January 20, 2005

Yuko Mitsui

PATENT OFFICE
JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy of the following application as filed with this Office.

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Application Number: Patent Application No. 2000-087501

Applicant(s): OLYMPUS OPTICAL CO., LTD.

This 27th day of September 2001

Commissioner,
Patent Office Kozo OIKAWA (seal)

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2000-087501

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[Title of the Invention]	METHOD FOR DETECTING SINGLE NUCLEOTIDE SUBSTITUTION USING FLUORESCENT CORRELATION SPECTROSCOPY
[Number of Claims]	4

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[Others] Patent application relating to results of a research sponsored by a nation or the like (the tenth year of the Heisei era, New Energy Resources·Industrial Technology Comprehensive Development Organization "Method for Detecting Single Nucleotide Substitution Using Fluorescent Correlation Spectroscopy", Sponsored Research, to which Article 30 of Special Measure Law for Revitalizing Industry is applied)

[List of Items Submitted]

[Name of Item] Specification 1

[Name of Item] Drawings 1

[Name of Item] Abstract 1

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[Necessity of Proof] Necessary

[Document] SPECIFICATION

[Title of the Invention] METHOD FOR DETECTING SINGLE NUCLEOTIDE SUBSTITUTION USING FLUORESCENT CORRELATION SPECTROSCOPY

[What is claimed is:]

[Claim 1] A method of detecting a single nucleotide substitution, comprising:

(1) hybridizing a test sample DNA containing a single nucleotide substitution with a plurality of types of DNA probes respectively having sequences complementary to a plurality of sequences that are supposed to be contained in the test sample DNA, and having a marker-substance labeled nucleotide corresponding to the single nucleotide replaced site; and

(2) optically measuring a change of the marker substance at a plurality of time points in the course of the hybridization.

[Claim 2] A method of detecting a single nucleotide substitution, comprising:

(1) hybridizing a test sample DNA containing a single nucleotide substitution with a plurality of types of DNA probes respectively having sequences complementary to a plurality of sequences that are supposed to be contained in the test sample DNA, and having a marker-substance labeled nucleotide corresponding to the single nucleotide replaced site; and

(2) reacting a nucleic acid synthetase having a repair function to a double stranded DNA strand, which is a part of the product obtained in (1); and

(3) optically measuring a change of the marker substance at a plurality of time points in the course of step (2).

[Claim 3] The method of detecting a single nucleotide substitution according to any one of claim 1 or 2, characterized in that the marker substance is a fluorescent substance.

[Claim 4] The method of detecting a single nucleotide substitution according to claim 3, wherein the optical measurement of a change of the marker substance is performed by measuring Brownian movement of the fluorescent substance according to a fluorescent correlation spectroscopy.

[Detailed Description of the Invention]

[0001]

[Technical Field of the Invention]

The present invention relates to a method of detecting a single nucleotide polymorphism (SNPs), and more particularly, to a method of detecting a single nucleotide substitution in DNA.

[0002]

[Prior Art]

The SNP (Single Nucleotide Polymorphism: hereinafter, referred to as SNPs) is defined as a polymorphism in which a single nucleotide of the nucleotide sequence is replaced. It has become obvious that the detection of a single nucleotide substitution is of essential importance in the screening of disease-related genes and gene diagnosis of diseases.

[0003]

As a method for detecting the single nucleotide substitution, a wide variety of methods are known including PCR-RFLP, PCR-SSP, PCR-SSO and the like. In these methods,

a PCR product is electrophoretically and hybridized with a solid-state probe sequence on a well plate, thereby performing the detection of the single nucleotide substitution. For example, in the PCR-SSP method, a sequence-specific primer reagent is used for specifically amplifying a polymorphism site. The method is frequently used for SNP determination. However, in this method, after the amplification, electrophoresis must be performed in order to confirm the presence of amplified product(s).

[0004]

Furthermore, another method is known for detecting SNP. In this method, SNP is detected by a VDA technique (high-density variant detection array) using a device such as a DNA chip or a DNA microarray (Science Vol. 280, May 15, 1998). In the VDA technique, a plurality of probe DNAs are densely arranged on the surface of a solid-phase substrate and sample DNA is allowed to hybridize with the probe DNA(s) on the solid-phase surface. However, the efficiency of this method is considered low, so that long time is required for reaction. In addition, since Tm value is hardly changed in the case of the single nucleotide substitution, it is difficult to accurately detect a mismatch by conventional methods.

[0005]

As described above, in any of the conventional methods, the operation is complicated and a large amount of samples are necessary, so that long time is required for the detection. For this reason, detection cannot be performed with an accuracy sufficient for clinical trials in conventional methods.

[0006]

[Objects of the Invention]

An object of the present invention is therefore to provide a method for sensitively and accurately detecting a single nucleotide substitution in a simple manner. Another object of the present invention is to provide a simple and easy-to-use detection method of a single nucleotide substitution, in which B/F isolation, PCR, electrophoresis, and the like are not required. Still another object of the present invention is to provide a method for detecting a single nucleotide substitution in which a plurality of polymorphism sites can be simultaneously detected by using small amounts of test sample and reagent.

[0007]

[Means for Achieving the Objects]

According to the present invention, there is provided a method of detecting a single nucleotide substitution, comprising:

(1) hybridizing a test sample DNA fragment containing a single nucleotide substitution with a plurality of types of DNA probes respectively having sequences complementary to a plurality of sequences that are supposed to be contained in the test sample DNA fragment, and having a marker-substance labeled nucleotide corresponding to the single nucleotide replaced site; and

(2) optically measuring a change of the marker substance at a plurality of time points in the course of the hybridization.

[0008]

[Embodiments of the Invention]

According to the present invention, there is provided a method for detecting a single nucleotide substitution in DNA. The method roughly comprises two steps, namely, a reaction step and a measurement step.

[0009]

The reaction step previously mentioned generally comprises, as described later in detail, hybridizing test sample DNA with a labeled DNA probe and treating the resultant double strand with an enzyme having a repair function.

[0010]

In the detection step, as described later in detail, a free micro-movement of molecules taking place in a reaction system of the reaction step is measured.

[0011]

1. Reaction step

Now, a reaction step of this example will be explained with reference to FIGS. 1 to 3. However, it is an example, so that it will not limit the present invention.

[0012]

(1) Test sample DNA

FIG. 1 is a schematic illustration of test sample DNA of this example. In this figure, a polymorphism gene of the test sample DNA has two polymorphism sequences, namely, the polymorphism is present as polymorphism I or polymorphism II. Polymorphism I and polymorphism II are homologous except a single-nucleotide replaced site. Furthermore, the

single-nucleotide replaced site of polymorphism I is adenine (hereinafter, referred to as "nucleotide (A)" or "A" as shown in the figure). The single-nucleotide replaced site of polymorphism II is guanine (hereinafter, referred to as "nucleotide (G)" or "G" as shown in the figure).

[0013]

(2) Labeled DNA probe

Now, a probe for detecting the single-nucleotide replaced site (shown in FIG. 1) is shown in FIG. 2. Probe I is composed of a sequence complementary to the sequence from the 3' end to the single-nucleotide replaced site of polymorphism I. In addition, a marker substance is attached to the nucleotide of the 3' end of probe I, (that is, the nucleotide paired with the nucleotide of the single-nucleotide replaced site). In this example, the 3' end of probe I is a labeled thymine (hereinafter, referred to as "(T)" or "T" as shown in the figure).

[0014]

Similarly, probe II is composed of a complementary sequence to the sequence from the 3' end to the single-nucleotide replaced site of polymorphism II. In addition, a marker substance is attached to the nucleotide of the 3' end of probe II. In this example, the 3' end of probe II is a labeled cytosine (hereinafter referred to as a "nucleotide (C)" or "C" as shown in the figure).

[0015]

(3) Reaction step

FIGS. 3 and 4 show a first aspect of the present

invention, which is the simplest example. In this example, it is determined that test sample DNA contained in a sample is either polymorphism I or II. In this case, it is assumed that the sample contains polymorphism I.

[0016]

Probes shown in FIG. 2 are placed separately in vessels varied depending upon types, and subjected to reactions with the sample. FIG. 3 shows a reaction performed in vessel I. To explain more specifically, in vessel I of FIG. 3, hybridization of polymorphism I with probe I proceeds under optimum conditions. As a result, polymorphism I is completely matched with probe I. A reaction performed in vessel II is shown in FIG. 4. More specifically, in vessel II of FIG. 4, polymorphism I is hybridized with probe II under optimum conditions. As a result, a mismatch is observed at the single-nucleotide replaced site.

[0017]

Subsequently, DNA polymerase having a repair function is added to each of the completely matched double strand and the mismatched double strand (FIGS. 3 and 4). As a result, the polymerase does not exhibit an activity (namely, 3' → 5' exonuclease activity) to the completely matched double strand of vessel I (FIG. 3). In contrast, the polymerase of vessel II recognizes a mismatch of the 3' end of the double strand formed of polymorphism I and probe II, and delete the mismatched nucleotide, and further extends the DNA strand (FIG. 4). With the enzymatic activity, the labeled nucleotide (C) is liberated in vessel II (FIG. 4).

[0018]

The free micro-movement of marker molecules obtained by the aforementioned reaction(s) is measured by the detection means described later. The free micro-movement of the target molecule can be varied depending upon the size of the marker molecule. Therefore, it is possible to obtain information on the nucleotide sequence by measuring the micro-movement of the marker molecules by using as the marker substance as an index.

[0019]

(4) Terminology

The term "single-nucleotide replaced site" used herein refers to a polymorphism site consisting of a single nucleotide, from which the single-nucleotide polymorphism is derived. According to an embodiment of the present invention, if the single-nucleotide replaced site is present either singly or in a plurality of numbers in the sequence of a single test sample DNA, it (or they) can be detected. When a plurality of single-nucleotide replaced sites are detected, a plurality of types of marker substances may be used.

[0020]

The term "a nucleic acid synthetase having a repair function" used herein refers to an enzyme which recognizes a mismatch at the 3' end of the nucleotide sequence partially double stranded, and deletes the mismatched nucleotide, and then, synthesizes a nucleic acid under appropriate conditions to complete the double strand. According to an embodiment of the present invention, examples of usable enzymes include nucleic acid synthetase. Preferably, Ex/Taq or La/Taq

manufactured in Takara Shuzo Co., Ltd is used, but not to limited to this.

[0021]

The term "marker substance" used herein refers to a marker emitting a signal with much the same intensity at any measuring time-points. For example, luminescent substances, fluorescent substances, magnetic substances, and radioactive materials are included. Note that a substance suitable for a means to be used in the detection step (described later) should be selected as the marker substance.

[0022]

The term "free micro-movement" or "micro-movement" used herein refers to the Brownian movement.

[0023]

(5) Another embodiment of the reaction step

The simplest example of the reaction step included in the detection method of the present invention is described above. However, various changes and modifications can be made. For instance, a plurality of marker substances may be used. A marker substance may be attached to a probe at not only the 3' end but also the 5' end. A plurality of probes may be reacted with a sample in a single vessel.

[0024]

Furthermore, after a mismatch is recognized or dissociated by a nucleic acid synthetase having a repair function, the nucleotide sequence can be extended from a cleaved site toward the 3' end. In this case, the extension may be performed by supplying a requisite substrate and

a reagent to be further required, under optimum conditions. Alternatively, the extension may be performed by a polymerase chain reaction (hereinafter referred to as PCR). However, the extension may not be always performed.

[0025]

Moreover, a polymorphism site contained in a sample may be amplified by, for example, a PCR technique before the aforementioned reaction(s) are performed.

[0026]

2. Measurement step

(1) Measurement principle

The detection of the marker molecules obtained in the reaction step mentioned above is carried out by the following step. The measurement step according to the present invention comprises microscopically observing the movement of the marker molecules in a micro field of view, converting a plurality of measurement data items into time-dependent statistical data, and obtaining a reaction curve of a hybridization reaction based on the statistical data. In the reaction curve, the initial height of the curve represents the number of nucleotide molecules.

[0027]

The measurement step of the present invention is performed in a three-dimensional micro field of view. By virtue of this, a free micro movement of the target molecules in a sample-containing solution can be measured with a high accuracy. Assuming that the measurement step is performed by carrying out the measurement in a two-dimensional field of

view, it is impossible to capture a three-dimensional free movement, such as the Brownian movement, of the marker molecules. As a result, the measurement accuracy becomes low. Therefore, such a measurement method is not preferable.

[0028]

Since the micro field of view employed in the measurement step is formed by a confocal optical system, measurement data having a deep depth-of-field can be obtained. By virtue of this, some of individual marker molecules always come into a focus in a field of view, so that an accurate portion and output data can be supplied to a measurement means.

[0029]

Since the micro field of view is a diffraction-limited region near a focal point, the individual marker molecules can be measured in a high S/N ratio.

[0030]

The diffraction-limited region is formed by a pin hole of 15 ± 5 μm in average diameter. Therefore, the measurement data of a small number of marker molecules selected can be efficiently obtained.

[0031]

A particularly preferable micro space is nearly a cylindrical region having an average diameter of 200 ± 50 nm and an average diameter on the optical axis of 2000 ± 500 nm. It is therefore possible to capture a free micro movement of the marker molecules brought into within the measurement field of view.

[0032]

In the measurement step of the present invention, the output signal intensity of marker molecules (one or more molecule) is measured in a predetermined space. Furthermore, the increase or decrease of the output signal intensity thus obtained is employed as an index. Based on variation of the output signal intensity, it is possible to obtain the moving speed of the marker molecules moving in or out of a micro measurement field of view. Therefore, the marker molecule to be labeled to a probe is preferably a marker material which can output a signal with a constant intensity at any time points. Examples of such a marker molecule include luminescent substances, fluorescent substances, magnetic substances and radioactive materials. As the luminescent substances and fluorescent substances, a dye capable of emitting luminescence or fluorescence for a long time is preferably selected. If a material containing a luminescent dye or fluorescent dye is used as the marker molecule, an optical determination of a molecular condition can be made at a molecular level by a device of a simple structure. Furthermore, when nucleotide molecules are complementarily bound to each other, use may be made of a substance capable of being intercalated in the complementary binding portion, thereby varying its fluorescent characteristics from those of a free state. Examples of such a fluorescent dye include acridine orange, thiazole orange, oxazole yellow, and rhodamine.

[0033]

When the positional change of a fluorescent molecule is

measured, fluorescence may be measured in the form of data by using a fluorescent measurement means such as a photomultiplier or a photodiode serving as a measurement means. Furthermore, the fluorescent measurement means may have a measurement mode capable of measuring a single photon in order to measure individual fluorescent molecules.

[0034]

In the present measurement step, the micro-movement of the target molecules can be accurately measured and by measuring the fluctuation movement of the marker molecules in a liquid. It is preferable that the measurement of the fluctuation movement be performed through the operation using the autocorrelation function (Autocorrelation function). When a fluorescent substance is used as the marker molecule, it is preferable that fluorescence correlation spectroscopy (hereinafter simply referred to as "FCS") be employed.

[0035]

As to the operation method for analyzing data obtained by measuring characteristics of a biological material by FCS, the report (Dinji, M., Rigler, R., Nucleic Acids Research 23, 1795-1799, 1995) can be referred to. The document reports the case where the hybridization reaction between a labeled nucleic acid probe and a target nucleic acid molecule is observed by FCS. FCS and operation of measurement data will be more specifically described later.

[0036]

If FCS is used, it is possible to obtain the number of fluorescent particles contained in an extremely small volume of

a sample and a diffusion constant almost in real time without a separation step. If the FCS is used, B/F separation is not required, so that the measurement time can be reduced. Since a solution system can be subjected as it is to the measurement, the measurement time can be further reduced. In addition, a biological molecule can be measured under natural conditions.

[0037]

(2) Measuring device

An example of a measuring device to be used in the measurement by FCS will be explained with reference to FIG. 5. An FCS device shown in FIG. 5 comprises an inverted fluorescent microscope 1 using a confocal optical system, a photomultiplier 2 for measuring fluorescence emitted from a sample, a data processing device 3 for receiving measurement data and calculating them by an autocorrelation function to convert them into numerical values or graphic plots, and a display unit 4 for displaying the operation results on a screen.

[0038]

A sample-containing solution 11 is easily set, as shown in FIG. 5, in such a manner that the solution 11 is dotted on a slide glass 13 placed on a sample base 12. Since a small amount of sample-containing solution is used in this device, a cover 14 is placed on the slide glass 13 in order to prevent a moisture content from vaporizing. A low light-transmissible material is preferably used as the cover 14. Air-tightness and light-tightness can be simultaneously ensured by the presence of the cover. It is further preferable that a material having a light reflectivity as low as possible be used as the inner

surface of the cover in order to prevent excitation light from reflecting. An objective lens 15 is arranged right under the portion of the slide glass 13 on which the sample-containing solution 11 is dotted so as to focus within the sample-containing solution 11. Note that the fluorescent microscope 1 may be a reflection type. In the reflection type, the sample-containing solution 11 may be dotted directly on the lower surface of the objective lens 15. In the example shown in FIG. 5, an argon (Ar) ion laser is used as a laser-generating device 16, which is a light source of the fluorescent microscope 1. Alternatively, a krypton-argon (Kr-Ar) ion laser, helium-neon (He-Ne) laser, helium-cadmium (He-Cd) laser may be used depending upon a type of a fluorescent substance. If necessary, various operations such as the loading/unloading of the slide glass 13 into/from the fluorescent microscope 1, dotting of a sample-containing solution onto the slide glass 13 and open/shut of the cover 14 may be performed automatically.

[0039]

FIGS. 6 (A) and 6 (B) are magnified views of a measurement portion of the fluorescent microscope 1 of FIG. 5. In FIG. 6 (A), a micro space 20 is formed which is defined by the positions of the slide glass 13 and the objective lens 15 having a predetermined aperture (FA = 1.2 in the figure). As shown in FIG. 6 (B), the micro space 20 is actually a focal point of laser light having a volume. The shape of the micro space 20 is nearly a cylindrical shape stretched up and down from a constricted middle portion (see FIG. 6B). The field of vision 20 is restricted by the length Z

on the optical axis and an average radium Y based on the reference position as a focal point. In the micro space 20, fluorescence of individual fluorescent molecules can be accurately measured. This is because the volume of the micro space 20 is reduced to the minimum sufficient to monitor the micro movement of the fluorescent molecules. With this structure, it is possible to remove the noise derived from the fluorescent molecules and present except the vicinity of the focal point of the sample-containing solution 11.

[0040]

When the fluorescence intensity of fluorescent molecules moving in and out of the confocal region of the confocal microscope (as shown in FIG. 6) having a length L and a width W is captured per molecule, the fluctuation of fluorescence intensity can be detected. Furthermore, when the fluctuation of the fluorescence intensity obtained in the form of data is converted by an autocorrelation function, statistical data is given. In this way, the number and sizes of fluorescent molecules can be detected without isolating the molecules. An example of data measured by the above device is shown in the form of a graph of FIG. 7. The graph of FIG. 7, the fluorescence intensity I(t) is plotted on the vertical axis and time (t) is plotted on the horizontal axis. When the data is converted by an autocorrelation function shown below, the graph shown in FIG. 8 is given.

[0041]

The autocorrelation function is

$G(\tau) : G(\tau) = \langle I(t)I(t + \tau) \rangle$

When this is normalized by the square of average intensity <I> and developed, the following approximation is given:

[0042]

[Chemical 1]

$$G(\tau)/\langle I\rangle^2 = C(\tau)$$

$$= 1 + \frac{1}{N}\left[\frac{1}{1+4D\tau/w_{xy}^2}\right]\left[\frac{1}{1+4D\tau/w_z^2}\right]\frac{1}{2}$$

[0043]

Where C(o)= 1+1/N in which $\tau = 0$, D = diffusion constant, N = the number of molecules in a solution.

[0044]

3. Another preferred embodiment

According to another aspect of the present invention, the embodiment mentioned above can be modified as described below. A marker substance different in fluorescent wavelength may be attached to the nucleotide molecule of the 3' end of the probe depending upon the type of probe. The probe thus prepared is mixed with a test sample DNA to perform hybridization in the same manner as in the embodiment described above. The nucleotide mismatched with the nucleotide sequence is enzymatically removed. Subsequently, detection is performed by FCS. At this time, individual marker substances are analyzed based on wavelengths different from each other. By the analysis, it becomes apparent which probe is completely matched with the nucleotide sequence. At the same time, the type of

the nucleotide at the 3' terminal is identified. It is there determined which type of polymorphism is present. As a result, the single nucleotide replacement can be more reliably detected. Furthermore, when another type of fluorescence is used, the wavelength of the excitation light may be changed. Alternatively, the wavelength of the detection light may be changed by attaching a filter to the detection portion.

[0045]

[Advantages of the Invention]

According to an aspect of the present invention, there is provided a single nucleotide detection method simply and quickly performed. Such an advantage is derived from the principle of the present invention originally found. More specifically, desirable detection of the present invention can be performed by labeling each of polymorphism-specific probes with a marker, reacting the probe with a test sample DNA, and detecting the marker. In this way, the reaction can be captured at a molecular level. At this time, the marker substance is measured at a plurality of time points, thereby detecting and evaluating the time-dependent positional change. Consequently, the positional change, which is varied depending upon the size of the molecule, can be quantitatively determined. It is therefore not necessary to carry out operation steps required by conventional methods including a B/F separation step, a reaction step of a substrate with an enzyme marker reagent, an exposure step of an RI marker reagent to a radiation-sensitive film, a PCR step, and an electrophoresis step.

[0046]

To explain more specific, conventional methods requires PCR and to extend the nucleotide sequence up to 200 to 300 nucleotides in order to obtain clear results from electrophoresis. However, electrophoresis and PCR are not always needed according to an embodiment of the present invention. If the PCR is performed to improve the reliability, a sufficiently reliable data can be obtained by extending the nucleotide sequence up to a length of 10 to 30 bases.

[0047]

The detection method according to an embodiment of the present invention can be performed by using extremely small amounts of test sample and reagent. More specifically, it is sufficient if the test sample is contained in the order of femto liter (fL). This is because the detection region of the signal to be emitted is a micro space having a size of 200 nm (diameter) × 2000 nm (axial length). Therefore, according to an embodiment of the present invention, it is possible to simultaneously measure a plurality of test samples by using extremely small vessels. Furthermore, it is possible to reduce the amounts of test sample and detection reagent, such as an enzymatic reagent, to be required for detection.

[0048]

Furthermore, according to an embodiment of the present invention, a plurality of reagents having specificities to different polymorphism sites can be mixed in the same vessel and therefore simultaneously applied to the test sample. As a result, not only the amount of sample DNA but also the number

of reaction vessels can be reduced. In addition, a polymorphism gene test can be easily performed. Moreover, the detection method according to the present invention can be performed at least with a mixture of a DNA sample and reagent, so that the entire system can be automated.

[0049]

According to an embodiment of the present invention, a single nucleotide substitution can be detected with a high sensitivity. Since the method of this example mentioned above is performed in a homogeneous system little affected by the background, the detection accuracy is high. This is based upon the detection principle of the present invention.

[Brief Description of the Drawings]

[FIG. 1]

A schematic illustration of test sample DNA detectable in a detection method according to the present invention.

[FIG. 2]

A schematic illustration showing a preferred example of a probe which can be used in a detection method according to the present invention.

[FIG. 3]

A scheme showing a detection method according to the present invention.

[FIG. 4]

A scheme showing a detection method according to the present invention.

[FIG. 5]

A scheme showing an example of a detection system which

can be used in a detection method according to the present invention.

[FIG. 6]

Views showing a measurement portion of a fluorescent microscope to be used in a detection method according to the present invention.

[FIG. 7]

A graph showing a time-dependent change of fluorescence intensity to be obtained in a detection method according to the present invention.

[FIG. 8]

A graph showing statistical data obtained by converting the data shown in FIG. 7 by an autocorrelation function.

[Explanation of Reference Symbols]

1 … Fluorescent microscope

2 … Photomultiplier,

3 … Data processing device,

4 … Display unit,

11 … Sample-containing solution,

12 … Sample base,

13 … Slide glass,

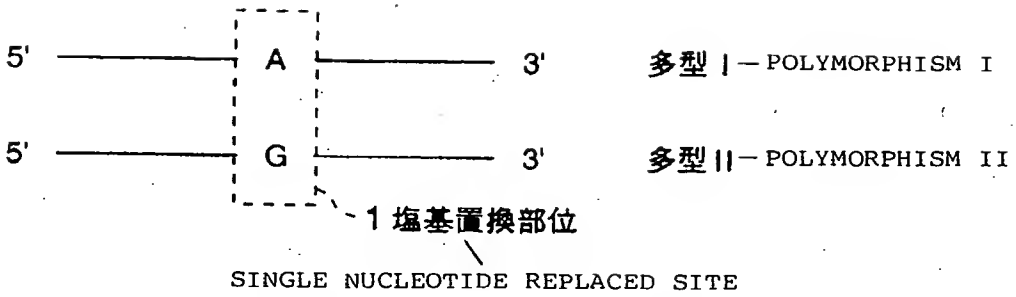
14 … Cover,

15 … Objective lens,

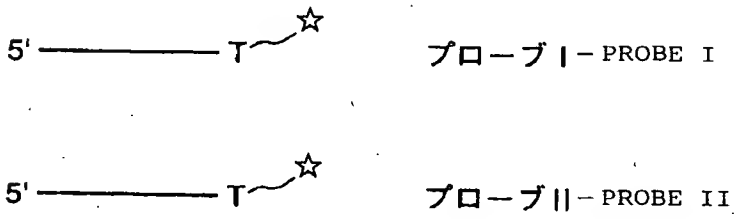
16 … Laser-generating device.

【書類名】 図面
[NAME OF DOCUMENT] DRAWINGS

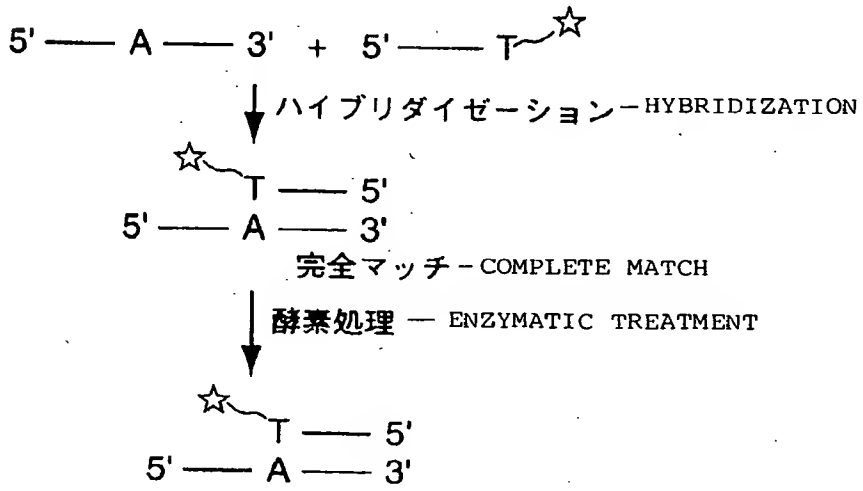
【図１】
[FIG. 1]



【図２】
[FIG. 2]

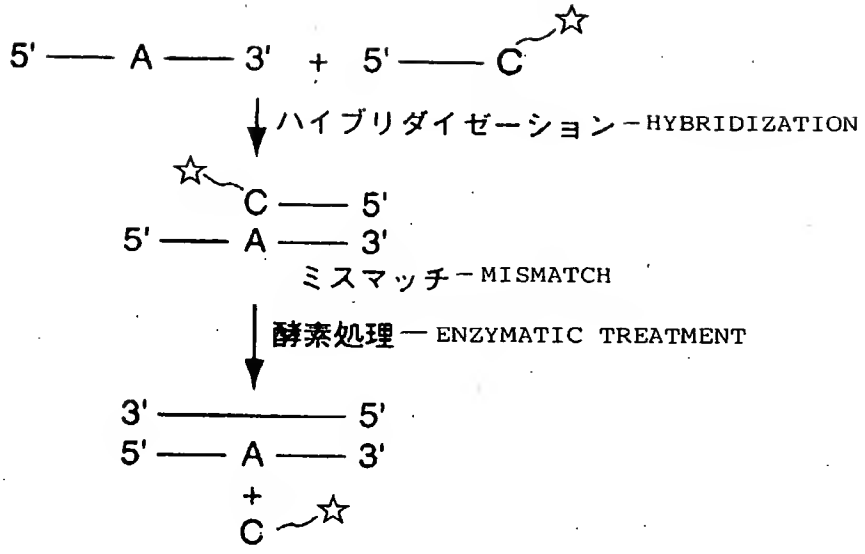


【図３】
[FIG. 3]



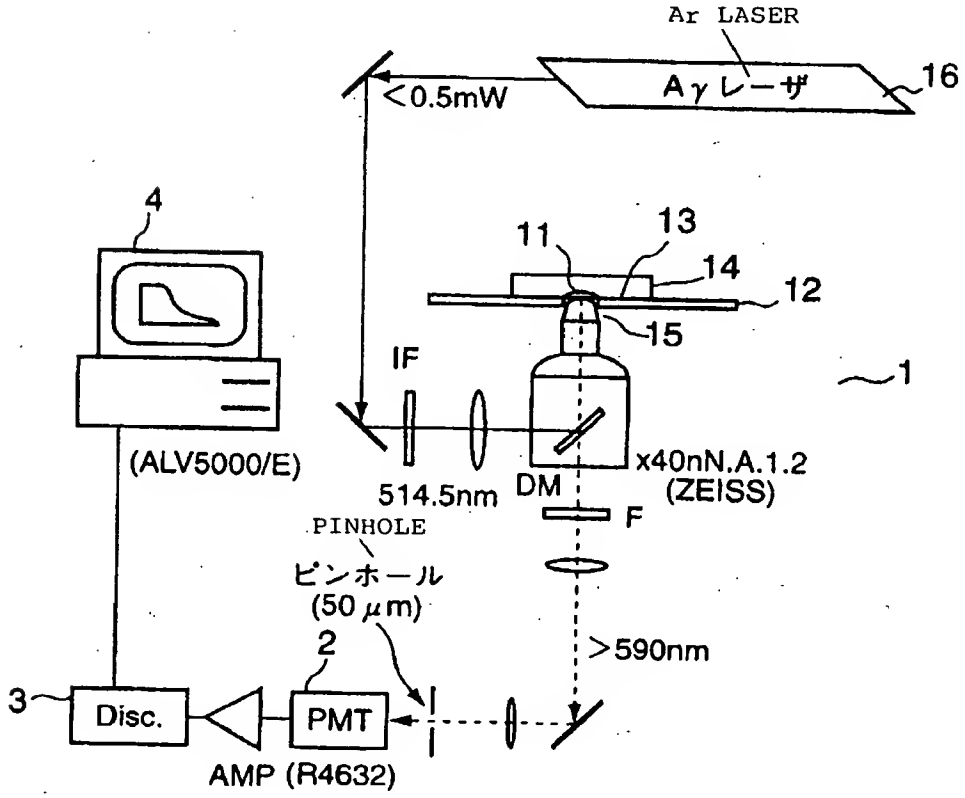
【図４】

[FIG. 4]



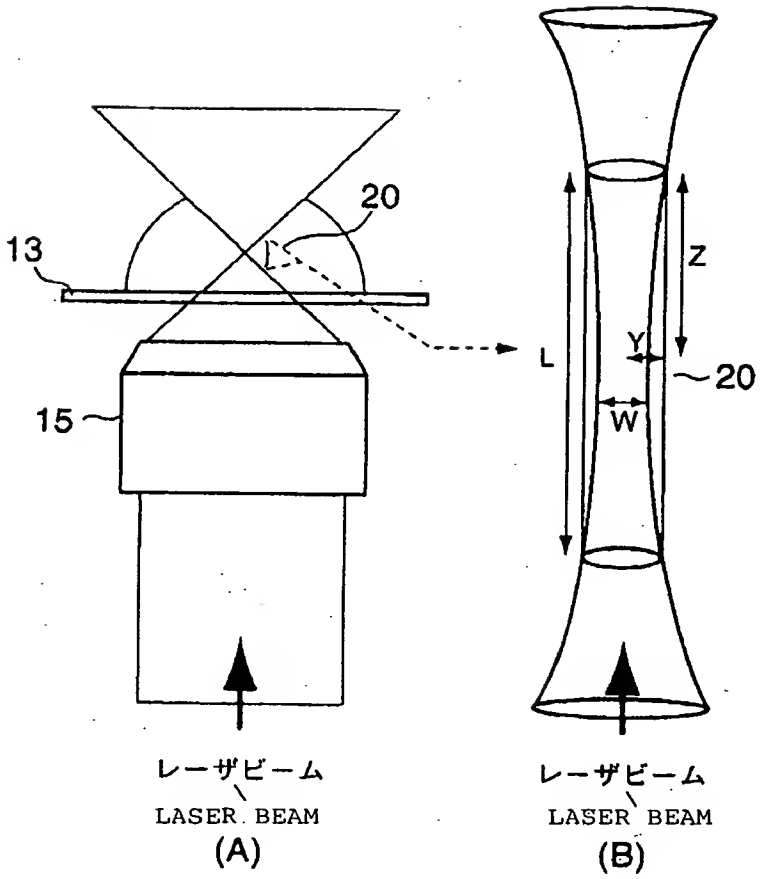
【図５】

[FIG. 5]



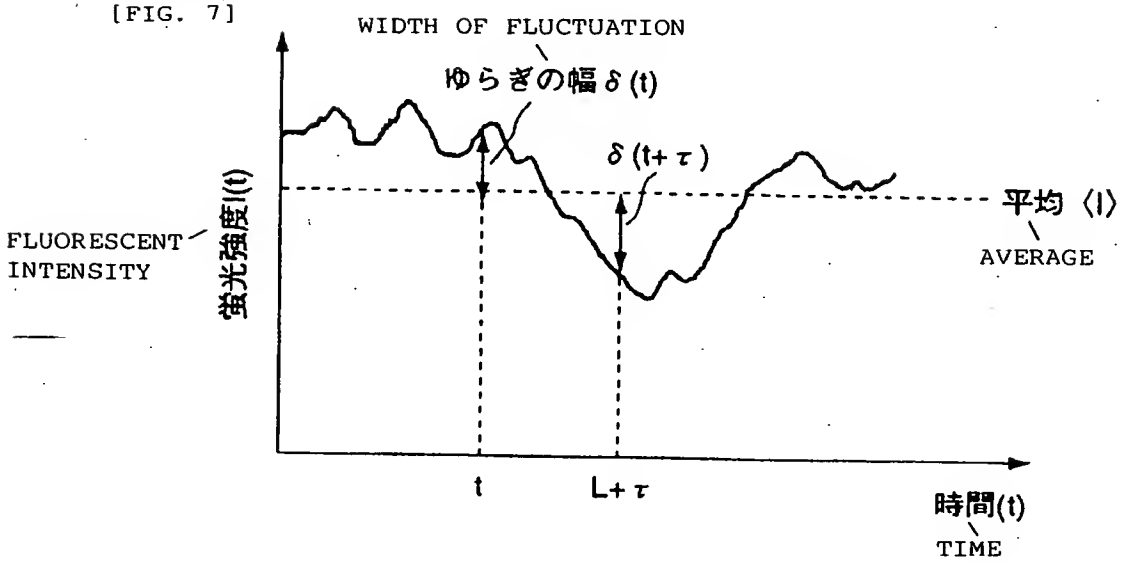
【図６】

[FIG. 6]



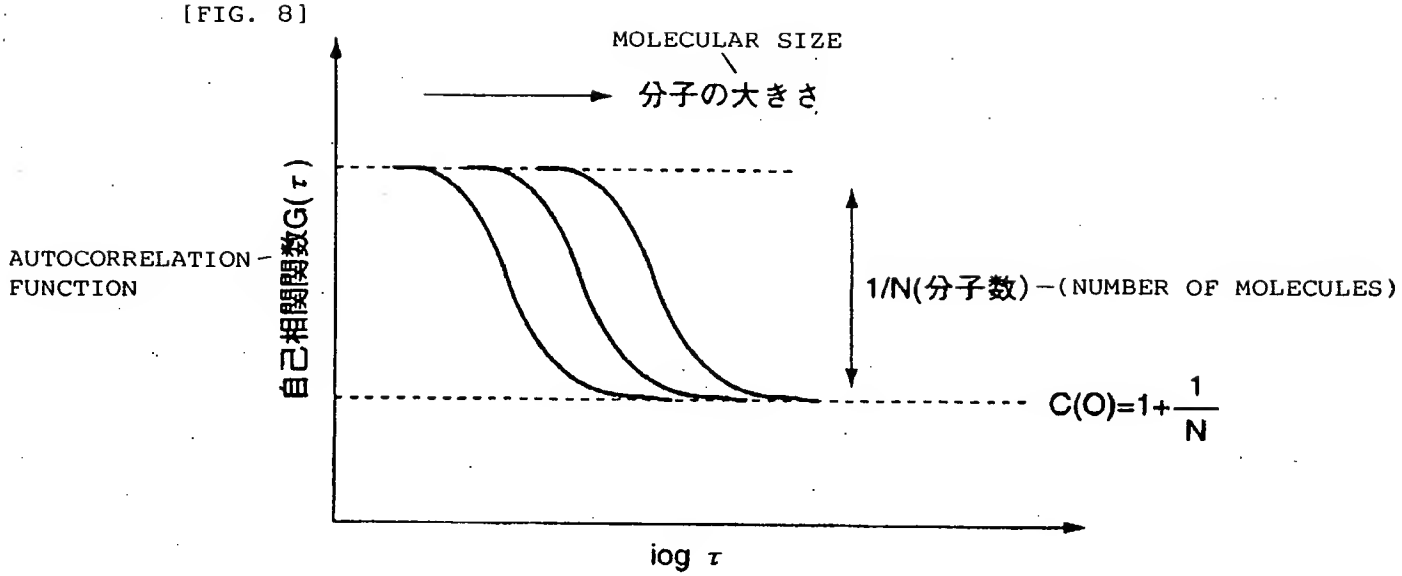
【図７】

[FIG. 7]



【図８】

[FIG. 8]


MOLECULAR SIZE
分子の大きさ
AUTOCORRELATION FUNCTION
自己相関関数G(τ)
1/N(分子数)－(NUMBER OF MOLECULES)
C(O)=1+1/N
log τ

[Document] ABSTRACT

[Abstract]

[Objects] An object of the present invention is therefore to provide a method for sensitively and accurately detecting a single nucleotide substitution in a simple manner. Another object of the present invention is to provide a simple and easy-to-use detection method of a single nucleotide substitution, in which B/F isolation, PCR, electrophoresis, and the like are not required. Still another object of the present invention is to provide a method for detecting a single nucleotide substitution in which a plurality of polymorphism sites can be simultaneously detected by using small amounts of test sample and reagent.

[Means for Achieving the Objects] A method of detecting a single nucleotide substitution according to the present invention comprises:

(1) hybridizing a test sample DNA containing a single nucleotide substitution with a plurality of types of DNA probes respectively having sequences complementary to a plurality of sequences that are supposed to be contained in the test sample DNA, and having a marker-substance labeled nucleotide corresponding to the single nucleotide replaced site; and

(2) optically measuring a change of the marker substance at a plurality of time points in the course of the hybridization.

[Elected Figure] NONE

APPLICANT'S PAST DATA

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[Reason for Change] New Registration

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